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1288356

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February 23, 2005

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APPLICATION NUMBER: ***60/540,566***
FILING DATE: ***January 30, 2004***
RELATED PCT APPLICATION NUMBER: ***PCT/US05/02745***

UNITED STATES PATENT AND TRADEMARK OFFICE
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as Express Mail (label number EV204080676US) in an envelope addressed to: Mail Stop Provisional Patent Applications, Commissioner For Patents, PO Box 1450, Alexandria, VA 22313-1450 on Janaury 30, 2004 under the provisions of 37 CFR §1.10.

Mailer's Name (Print/Type)	Katrina Holland	Signature	Katrina Holland	Date	12/19/03

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Harold J.	Schreier	Baltimore, MD
Yossi	Tai	Rchovot, Israel

[ ] Additional inventors are being named on the _____ separately numbered sheets attached hereto.

TITLE OF THE INVENTION (280 characters max)

DISSIMILATORY SULFATE REDUCTION AS A PROCESS TO CONTROL NITRATE LEVELS IN MARINE RECIRCULATION AQUACULTURE SYSTEMS

Direct all correspondence to: CORRESPONDENCE ADDRESS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

[x] Specification *Number of Pages* 6   [ ] CD(s), Number [ ]

[x] Drawing(s) *Number of Sheets* 2   [ ] Other (specify) [ ]

[ ] Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[x] Applicant claims small entity status. See 37 CFR 1.27.

[ ] A check or money order is enclosed to cover the filing fees.

FILING FEE AMOUNT ($)80.00

[X] The Commissioner is hereby authorized to charge deficiencies in filing fees or credit any overpayment to Deposit Account Number: 083284

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[ ] No.

[ ] Yes. the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE Marianne Fuierer

TYPED or PRINTED NAME Marianne Fuierer

DATE 1/30/04

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Effective 10/01/2003. Patent fees are subject to annual revision.

[X] Applicant Claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	NA
Filing Date	January 30, 2004
First Named Inventor	Schreir, et al.
Examiner Name	NA
Art Unit	NA
Attorney Docket No.	4115-197 PRV

METHOD OF PAYMENT (*check all that apply*)

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1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($)

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fee from below		Fee Paid
Total Claims		-20**=		X		=	
Independent Claims		- 3**=		X		=	
Multiple Dependent						=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	**Reissue independent claims over original patent
1205	18	2205	9	**Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)80.00

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FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge – late filing fee or oath	
1052	50	2052	25	Surcharge – late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive – unavoidable	
1453	1,330	2453	665	Petition to revive – unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) ____________________

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

SUBMITTED BY				Complete (*if applicable*)	
Name (Print/Type)	Marianne Fuierer	Registration No. (Attorney/Agent)	39983	Telephone	(919) 419-9350
Signature	Marianne Fuierer			Date	

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This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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UNIVERSITY OF MARYLAND BIOTECHNOLOGY INSTITUTE (UMBI)

DISCLOSURE AND RECORD OF INVENTION

This invention was made and/or reduced to practice in the course of work performed at the UMBI facility.

Mail completed disclosure to UMBI Office of Research & Development,

701 East Pratt Street, Suite 200, Baltimore, MD 21202

Telephone (410) 385-6328, if you have questions regarding this form.

Title of Invention:
Dissimilatory Sulfate Reduction as a Process to Control Nitrate Levels in Marine Recirculated Aquaculture Systems.

Please list names of those who contributed to the inventive thought and to the final result of the invention. If a patent application is filed the Office of Research & Development will make a legal determination of inventorship.

Contributor(s): Full Name	Title/Position	Employer	Division	Phone No.	Mail Stop
Yossi Tal	Postdoctoral Research Associate	UMBI	COMB	410-234- 8875	
Harold J. Schreier	Associate Professor	UMBI	COMB	410-234-8874	

Contributor(s) Permanent Home Address(es):

Name	Citizenship	Street Address	City, State, and Zip Code
Yossi Tal	Israel	4/7 Hag'ra	Rehovot, Israel
Harold J. Schreier	U.S.A.	2400 Sugarcone Road	Baltimore, MD 21209

IMPORTANT - Check the funding source for this Invention & provide the information requested:

___Government Grants
Funding Agency and Grant No.___

___Principal Investigator ___

___Sponsored Research Grants
Sponsor Name___
___Principal Investigator ___

___Private, Non-Government Grants___
Funding Agency and Grant No. ___
Principal Investigator ___

___Subcontract
Subcontract No. ___ Contractor ___

__Other
Describe ___

From what source do you reasonably expect future or continuing funding?

Source BARD ___ Is this ___ continuing funding or X future funding?

___ No future funding is currently expected.

Conception (Date, Place):

Earliest documentation of your invention: (please provide date and identify the document)

First Sketch or Drawing: [redacted]

First Written Description:

Names of witnesses or others with knowledge of facts relating to conception:

Steve Rodgers

Reduction to Practice:

Date first model completed: [redacted]

Date of operation and testing: [redacted]

Place of test: Aquaculture Research Center, COMB

Results of testing:

Witnesses or others with direct knowledge of test:

Steve Rodgers, Allen Place, Yonathan Zohar, John Stubblefield

Important: WAS ANY PROPRIETARY MATERIAL FROM OUTSIDE YOUR LABORATORY USED TO DEVELOP YOUR INVENTION (e.g., software code, cell line, antibody, DNA fragments, or chemical compound)? If yes, please describe.

ARC systems 8-1, 8-2, 8-3 and 8-4 were used to develop and test the invention.

DESCRIPTION:

Background of the invention   Please summarize:

1) Technical problems overcome to make the invention,
2) What your invention enables people to do that couldn't be done as well before,
3) How people currently address the problem your invention addresses.

See Attached.

Summary of the Invention   Please include a sketch of the invention if possible (you may attach extra pages):

See Attached.

List uses of the invention - research, commercial, pilot plant, etc.; Think as broadly as possible.
Identify companies that might be interested in licensing this technology.

Because the invention establishes an anaerobic environment to carry out dissimilatory sulfate reduction using organic waste produced by fish, it should be applicable for ANY marine recirculated system.

Publications (including UMBI reports, meeting abstracts, or prior patents or patent applications) that ACTUALLY DESCRIBE or RELATE to the invention:

Title/Subject	Publication Date	Indicate "Describes" or "Relates" to Inv.	Journal and/or UMBI Rep. No. If prior UMBI pat. appl., Disclosure ID#

Papers you have SUBMITTED or are PREPARING for publication that describe or relate to your invention:

Title/Subject	Anticipated Publ. Date	Indicate "Describes" or "Relates" to Inv.	Journal and/or UMBI Rep. No. If prior UMBI pat. appl., Disclosure ID #

An Abstract, entitled "Dissimilatory Sulfate Reduction as a Means to Control Nitrate Levels in Marine Recirculated Aquaculture Systems," was submitted [redacted] to the Fifth International Conference on Recirculating Aquaculture for consideration. This Abstract describes the invention.

Please notify the Office of Research & Development(x 6328) if you later plan new publications that describe or relate to the invention.

I believe myself to have contributed to be the above-described invention. (Each contributor must sign.)

CONTRIBUTOR: ____________ DATE: ______

WITNESS: ____________ DATE: ______

CONTRIBUTOR: ____________ DATE: ______

WITNESS: ____________ DATE: ______

CONTRIBUTOR: ____________ DATE: ______

WITNESS: ____________ DATE: ______

Read and Understood by:

____________ Date: ______

UMBI Office of Research & Development Reviewer

____________ Date: ______

Center's Director

Background and Summary:

The ability to effectively manage nutrient wastes in recirculating marine aquaculture systems is key to efficiently control these systems and limit the amount of water exchange necessary to maintain a high degree of water quality. Nitrogenous wastes are eliminated through the action of nitrifying and denitrifying biofilter units that rely on oxygen and reduced organic compounds, respectively, for their activity. In a previous invention disclosure (Provisional U.S. Patent Application No. 60/335,024) we described the stimulation of denitrification in a recirculated aquaculture bead biological filter by the addition of a slow-release carbon source. For that application, the carbon source (glucose polymers) was necessary to provide reducing equivalents to both decrease oxygen availability (making the system anaerobic) and driving the denitrification process, as exemplified in the following equation:

$$\text{Glucose} + 4.8\ NO_3^- + 4.8\ H^+ \rightarrow 6\ CO_2 + 2.4\ N_2\ \text{(gas)} + 8.4\ H_2O \qquad \Delta G^{0'} = -2660\ \text{kJ}$$

For this process, nitrate (NO_3^-) is provided by the oxygen-dependent nitrification reaction that occurs within the aerobic compartment of the biofilter and is a result of oxidation of ammonia (NH_4^+), the metabolic waste product of fish, to nitrate via a nitrite (NO_2^-) intermediate, as follows:

$$NH_4+ + 1.5\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad \Delta G^{0'} = -274\ \text{kJ}$$

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \qquad \Delta G^{0'} = -74.1\ \text{kJ}$$

Bacteria are responsible for both nitrification and denitrification processes and we have demonstrated the presence of both nitrifying and denitrifying organisms in our system.

A major goal of developing an efficient wastewater filtration system is to decrease the frequency and amount of water exchanges necessary to maintain water quality. Decreasing this exchange not only saves on costs associated with replenishing water and salt (for marine systems) but also minimizes the level of nitrogen-rich waste that would be discharged into the environment. One significant source of water loss occurs during the removal of organic waste matter that arises from both fish excretion and uneaten food. Organic material is generally removed mechanically to avoid their consumption by bacteria, which dissipates oxygen and results in the buildup of toxic ammonia and noxious gases, such as hydrogen sulfide. This process is examplifed by the following reaction, where acetate (CH_3COO^-) is produced from the breakdown of fatty acids and sugars and sulfate (SO_4^{2-}) is provided as a major divalent ion of sea water:

$$CH_3COO^- + SO_4^{2-} + 3\ H^+ \rightarrow 2\ CO_2 + H_2S + 2\ H_2O \qquad \Delta G^{0'} = -57.5\ \text{kJ}$$

In this invention, we describe the potential of using the reducing capacity derived from organic waste degradation to drive denitrification in a marine recirculating fish system. We rely on coupling dissimilatory sulfate reduction to denitrification, as shown in the following equations:

Coupling dissimilatory sulfate reduction to denitrification:

$$5\ H_2S + 8\ NO_3^- \rightarrow 5\ SO_4^{2-} + N_2 + 4H_20 \qquad \Delta G^{0'} = -3721.5\ \text{kJ}$$

The system was established as follows (see accompanying diagram):

Two 4.2 m^3 tanks were operated with gilthead seabream, *Sparus aurata*, at a density of 10-50 kg/m^3 and a feeding rate of 1%-1.6% body weight/day. The tanks were connected to a 2 m^3 nitrifying moving bed bioreactor (MBB) with 1m^3 of polyethylene beads with specific surface area of 500 m^2/m^3 (4.86 cm^2/bead). A flow rate of 8 m^3/hr was set to enable two exchanges of tank water per hour through the filter. Attached to this tank as a side loop was a 0.3 m^3 cylindrical up-flow fixed bed biofilter filled with 0.2 m^3 of polyethylene beads for denitrification. This anaerobic biofilter component was set with a low flow rate of 0.1 m^3/hr (this set-up was described in Provisional U.S. Patent Application No. 60/335,024). Sludge (organic waste material) collection was carried out through a drum screen filter with backwash system that used tank water. Sludge and backwash water were collected in a 0.3 m^3 rectangular tank with 0.1 m^3 beads that provided a means for solids removal as well as substrate for bacterial colonization. Water from the sludge tank was pumped back into the system via the anaerobic biofilter and high-density sludge was collected and removed weekly. Ammonia, nitrite and nitrate levels as well as sulfate, sulfide, COD, pH and oxygen were monitored in each compartment. Our results indicated that the sludge/denitrifying filter sequence was very effective in stimulating nitrate removal. The denitrifying compartment removed as much as 40-70% of the nitrate load introduced from the system, which allowed for overall system nitrate concentrations to be maintained between 35-65 mg (NO_3-N)/l during operation and enabled daily water exchange to be less than 1% of total system volume. Stimulation of nitrate reducing activity was likely due, in part, to the presence of dissimilatory sulfate reduction activity that occurred during sludge waste decomposition; sulfide levels within the sludge compartment were as high as 60-80 mg/l and the redox potential reached values lower than -500 mV. On the other hand, effluent water from the denitrifying compartment showed no measurable sulfide and a redox potential between -50 and +100 mV.

We believe that our invention allows for denitrification to be driven by the redox gradient between system compartments resulting in sulfate reduction (sulfide production), which, in turn, was utilized for nitrate reduction. This process has wide application for marine recirculating systems, where sulfate concentration is not a limiting factor and minimizing water exchange is critical.

Results for first 49 days of operation are shown in Figure 1.

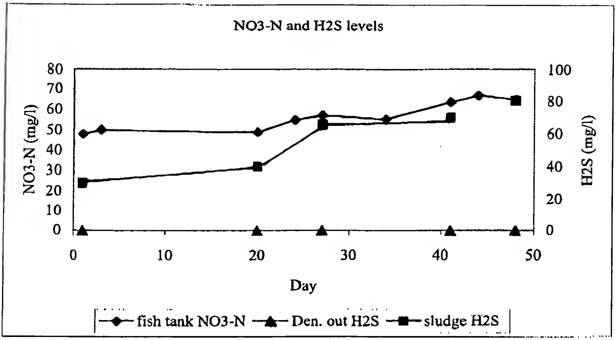

Figure 1. Nitrate-N levels for the tank system and levels for sulfide measured at the denitrifying compartment outlet (Den. Out) and sludge compartment.

Sulfide/Denitrification Coupled System